methods

A procedure for preparing oxazolines of highly unsaturated fatty acids to determine double bond positions by mass spectrometry

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Abstract **A convenient, mild, reliable method has been developed for preparing oxazolines of fatty acids and for using these derivatives to determine double bond locations in long-chain polyunsaturated and polyconjugated fatty acids. Fatty acyl mixed anhydrides are prepared using isobutylchloroformate and then converted to their ethanolamides by treatment with ethanolamine. Ethanolamides are subsequently cyclized to the corresponding oxazolines in ⩾85% yields by treatment with trifluoroacetic anhydride under mild conditions (>50° for 30–60 min). This general protocol can also be used to synthesize 4,4-dimethyloxazoline and benzoxazole derivatives of fatty acids. Gas chromatography-mass spectrometry of oxazoline derivatives of fatty acids yields prominent ions diagnostic of the structures of the parent fatty acids and, in the case of unsaturated fatty acids, indicating the positions of the double bonds.** **The utility of the method is illustrated with several fatty acids, including the conjugated 4E,6E,8E,10E,13Z,16Z,19Z-docosaheptaenoic acid.**—Kuklev, D. V., and W. L. Smith. **A procedure for preparing oxazolines of highly unsaturated fatty acids to determine double bond positions by mass spectrometry.** ***J. Lipid Res.*** **2003.** 44: **1060–1066.**

Supplementary key words arachidonic acid • docosahexaenoic acid • conjugated fatty acids

About 20 years have passed since the first application of 4,4-dimethyl-2-oxazolines of fatty acids as useful derivatives for mass spectrometric analysis (1, 2). The utility of these adducts for structural investigations of natural and artificial fatty acids is now well established (3), and several new protocols for their synthesis from 2-amino-2-methyl-1-propanol and different derivatives of fatty acids have been developed (4–6). Dimethyloxazoline derivatives, together with pyrrolidides and picolinyl esters, are currently the most widely used compounds for fatty acid structural investigations (7, 8). The "oxazoline technique" (3) has employed 4,4-dimethyloxazoline derivatives of fatty acids almost exclusively. The properties of these derivatives that are the basis for their widespread use include stable chromatographic behavior, high volatility, intense ion current, and formation of relatively simple mass spectra (3). Benzoxazoles derived from fatty acids possess similar advantages for structural studies by gas chromatography-mass spectrometry (GC-MS), but have not been widely used (9).

Two disadvantages of the current procedures for using dimethyloxazolines are that there is often incomplete reaction during the preparation of fatty acid derivatives, and that an intermediate appears that elutes somewhat later upon GC, but that gives a mass spectrum almost identical to that of the expected derivative (6). Additionally, the prolonged high temperature required for the preparation of dimethyloxazoline derivatives presents a problem, particularly with polyunsaturated fatty acids and other compounds with potentially labile functional groups. For example, *trans*-3-hexadecenoic acid, a fatty acid common in plant photosynthetic tissue, largely isomerizes to *cis*-2-hexadecenoic acid under conditions commonly used to form dimethyloxazoline derivatives (10), and crepenynic acid undergoes an unusual cyclization reaction (6). Even the milder derivatization technique described by Christie (6) leads, in the case of polyconjugated compounds, to degradation products and not oxazolines. A final problem associated with dimethyloxazoline derivatives is the presence of ring methyl groups that compromise the use of these derivatives in analyzing methyl-branched fatty acids (3).

In investigating new polyunsaturated and polyconjugated ω3 fatty acids (i.e., 4E,6E,8E,10E,13Z,16Z,19Z-docosaheptaenoic acid and 5E,7E,9E,11E,14Z,17Z-eicosahexaenoic acid), we encountered three problems in employing the commonly used techniques for GC-MS analysis. The first was connected with lability of the fatty acids under the derivatization conditions used to prepare dimethyloxazolines and picolinyl esters; the second was one-

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Abbreviations: GC-MS, gas chromatography-mass spectrometry; THF, tetrahydrofuran.

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column degradation of some of the derivatives (e.g., pyrrolidides) during GC-MS analysis; and the third was the uninformative mass spectra obtained with methyl esters resulting from isomerization caused by double-bond migration upon ionization. Thus, it became necessary to develop protocols that would provide milder conditions for the synthesis of stable volatile derivatives that would be useful for GC-MS structural analyses. Because it is well established that dimethyloxazolines of fatty acids have many advantages for characterizing highly unsaturated fatty acids by GC-MS (7, 8), we chose to modify and refine protocols for the use of this general type of derivative.

MATERIALS AND METHODS

Materials

All fatty acids were purchased from the Sigma Chemical Co. (St. Louis, MO) with a purity greater than 96%. Trifluoroacetic anhydride, 2-amino-2-methyl-1-propanol, ethanolamine, isobutylchloroformate, and thionyl chloride were products of Aldrich Chemical Co. (Milwaukee, WI) with a purity of at least 96%. Benzene, *n*-hexane, ether, and acetonitrile were distilled over phosphorus pentoxide, and tetrahydrofuran (THF) and triethylamine were distilled over metallic sodium before use. Silica gel "Selecto" 32–63 mm was purchased from Selecto Scientific (Suwanee, GA). Thin layer chromatography (TLC) plates were from Sigma Chemical Co. Various fatty acid reactants and products were visualized following TLC by spraying the plates with 5% phosphomolybdic acid in methanol followed by heating 2–3 min over a hot plate at 100°C.

Equipment

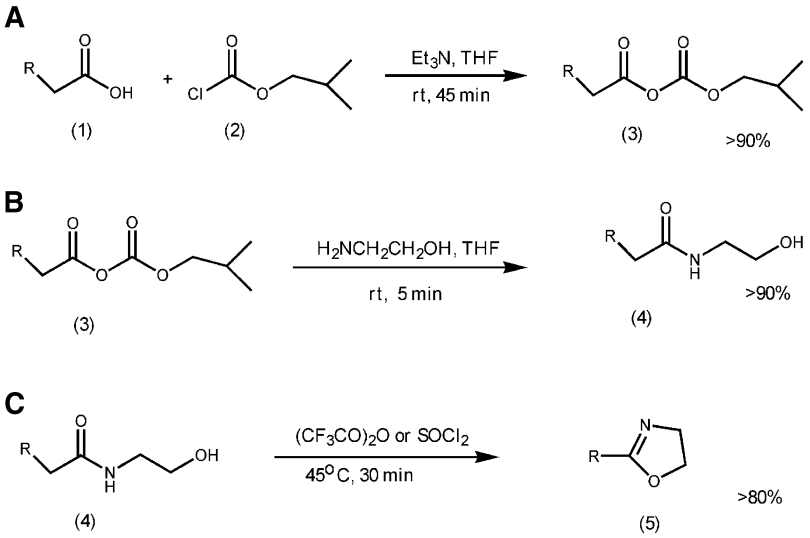
GC-MS was performed using a Hewlett-Packard 5890 gas chromatograph coupled to a Hewlett-Packard 5970 mass selective detector and a Hewlett-Packard 7946 computer. For GC, helium was used as the carrier gas at a flow rate of 35 cm/sec, the oven temperature was maintained at 210°C, and the injector and interface temperatures were 250°C. All GC separations were performed using capillary DB-5ms columns (30 m × 0.32 mm, 1 μm; Agilent J & W Scientific) with a constant injector split ratio of 1:60. Mass detector conditions were as follows: electron energy 70 eV, emission current 0.8 mA, accelerating voltage 8 kV, scale from 50 to 1,000. All ^{1}H-NMR spectra were recorded using a Varian INOVA-300 NMR operated at 300 MHz. For samples dissolved in $CDCl_3$, tetramethylsilane was used as the internal standard. All signal assignments were based on selective decoupling experiments.

Methods

Ethanolamide synthesis from fatty acids and ethanolamine. To a solution of 1 mg of a fatty acid 1 (**Scheme 1**) or a mixture of fatty acids in 0.2 ml of THF in a microvial (1–3 ml vol) was added 0.2 ml of a solution of 1 mg of triethylamine in THF. The reaction mixture was shaken vigorously, and 1 mg of isobutylchloroformate 2 (Scheme 1) in 0.2 ml of THF was added. The reaction mixture was maintained for 30 min at room temperature and after that evaporated under a stream of nitrogen. (The temperature may vary but the best yields were observed in the temperature range of −5°C to 30°C for reaction times of 30–60 min.)

Partial purification of the mixed anhydride. Partial purification of the fatty acid mixed anhydride 3 (Scheme 1) from the reaction mixture is not obligatory but was typically performed as follows to minimize by-products in the second step. A dry residue with the appearance of a white crystalline solid was obtained after complete evaporation of the reaction mixture. It was dissolved in dry *n*-hexane (0.3 ml) and filtered through glass wool prewashed with *n*-hexane, and the clean filtrate with the salt of triethylamine removed was collected and evaporated under a stream of nitrogen.

Synthesis of ethanolamides. The dry oily residue of the purified mixed anhydride 3 (Scheme 1) was dissolved in 0.30 ml of THF, and 1 mg of ethanolamine in 0.20 ml of THF was added. As judged by visualization of the reactants and products on TLC, the reaction is complete in 1–2 min if dry solvents and reagents are used. After 15 min, the reaction mixture was evaporated under a stream of nitrogen and used without purification for preparation of oxazolines. Ethanolamides of fatty acids are viscous oils or solids depending on the degree of unsaturation and have R_f values of ~0.5 in *n*-hexane-acetone (1:1, v/v).



Scheme 1. Synthesis of a fatty acid oxazoline. Details of the synthesis are presented in the text. Et_3N, triethylamine; THF, tetrahydrofuran.

Cyclization of ethanolamides to oxazolines. The ethanolamides of fatty acids 4 were dissolved in 0.3–0.4 ml trifluoroacetic anhydride and transferred into either an ampule or a reaction vial equipped with tightly fitting lid. The ampule was sealed or the vial was closed tightly, and the reaction mixture was kept at 45°C for 30 min. The reaction mixture was cooled to room temperature and volatile materials evaporated under a stream of nitrogen. The dry oily residue of oxazoline product 5 (Scheme 1) was dissolved in *n*-hexane and purified by microcolumn chromatography (~0.3 g of SiO_2 in a 1 ml pipette tip) eluting with a mixture of *n*-hexane-acetone (90:10, v/v). The eluate was collected and evaporated under a stream of nitrogen yielding a colorless oily product. The product was dissolved in 1 ml of *n*-hexane and analyzed by GC-MS. All of the oxazolines synthesized are colorless or pale yellow (in the case of highly unsaturated fatty acids) oils with a characteristic odor.

RESULTS AND DISCUSSION

2-Acyloxazolines were prepared from a variety of fatty acids and mixtures of fatty acids using protocols detailed in the Materials and Methods section and illustrated in Scheme 1. When dry solvents and good-quality reagents were used, the yields, determined gravimetrically, were ⩾75%, calculated on the basis of starting fatty acid. Among individual natural fatty acids, the procedure was used successfully to synthesize 2-acyloxazolines of stearic (18:0), oleic (18:1, n-9), linoleic (18:2, n-6), α-linolenic (18:3, n-3), dihomo-α-linolenic (20:3, n-3), arachidonic (20:4, n-6), eicosapentaenoic (20:5, n-3), and docosahexaenoic (22:6, n-3) acids. We also applied this technique to the synthesis of mixtures of 2-acyloxazolines from artificially created mixtures of saturated and monounsaturated fatty acids containing *a*) palmitic (16:0), stearic, arachidic (20:0), and behenic (22:0) acids; and *b*) palmitoleic (16:1, n-9), oleic, eicosenoic (20:1, n-9), and erucic (22:1, n-9) acids. Finally, several modified fatty acids were converted to the oxazolines, and among these were β-parinaric, (4E,6E,8E,10E, 13Z,16Z,19Z)-docosaheptaenoic, and (5E,7E,9E,11E,14Z)-eicosapentaenoic acids. In the following sections, we highlight features of the synthesis and describe the mass spectra of representative 2-acyloxazolines. Although not presented in detail here, we note that the approach used in this report for the synthesis of oxazoline derivatives was also found to be effective in synthesizing 4,4-dimethyloxazolines and benzoxazoles from a variety of fatty acids.

Synthesis of 2-acyloxazolines

The conversion of any fatty acid into an oxazoline requires the formation of an amide from the corresponding fatty acid and an aminoalcohol (Scheme 1, reactions a and b). A key feature of our protocol is the rapid formation of fatty acid mixed anhydrides and, subsequently, ethanolamides under very mild conditions (11–13). Using this approach, we were able, for the first time, to synthesize ethanolamides and, consequently, oxazolines from highly unsaturated and conjugated fatty acids such as docosaheptaenoic and parinaric acids. In contrast to chloroanhydrides, mixed anhydrides of fatty acids are relatively stable and can be purified, filtered, and evaporated to dryness without detectable degradation. The absence of by-products under the conditions described is an important characteristic of this reaction that has permitted it to be applied previously to prostaglandins and other labile eicosanoids (11–13). The reaction to form the amide between the mixed anhydride and amine in THF, acetonitrile, or methylene chloride proceeds rapidly (within several minutes) and almost quantatively (as judged by TLC of the reactants and products) to form the corresponding amides. We have successfully applied this technique to the synthesis of amides of fatty acids with ethanolamine, 2-amino-2-methyl-1-propanol, *O*-aminophenol, pyrrolidine, and several others. Following completion of the reaction, the sample can be evaporated to dryness under a stream of nitrogen at room temperature and used without further purification. Currently, the three most commonly used techniques for preparing fatty acyl amides are: *i*) activation of a fatty acid by formation of a chloroanhydride with subsequent reaction with an amine; *ii*) high-temperature aminolysis of a fatty acid ester (180–220°C); and *iii*) condensation using polyphosphoric ether as a condensing agent at elevated temperatures (70–90°C). The mixed anhydride procedure described here offers the major advantages of rapid reaction, mild conditions, and the use of inexpensive, readily available reagents.

At the present time, the four most common protocols for cyclization of hydroxyamides of fatty acids to form oxazolines are: *i*) heating at relatively high temperatures (⩾180°C) for 2–18 h (14); *ii*) the classsical treatment with thionylchloride (1, 2); *iii*) treatment with polyphosphoric ether and heating at about 70°C for 45–60 min (9); and *iv*) treatment with trifluoroacetic anhydride and heating at a temperature below 50°C for 30–60 min (6). The trifluoroacetic anhydride method proved to be the method of choice for working with polyunsaturated and especially polyconjugated fatty acids. To prevent formation of undesirable colored by-products, the reaction temperature must not exceed 50°C. Excess trifluoroacetic acid anhydride can be removed under a stream of nitrogen at room temperature, and the dry oxazoline redissolved and used for GC-MS without purification; however, purification by preparative TLC or by filtration through silica gel or Florisil with *n*-hexane-acetone (10:1, v/v) as the eluant eliminates most of the contaminants.

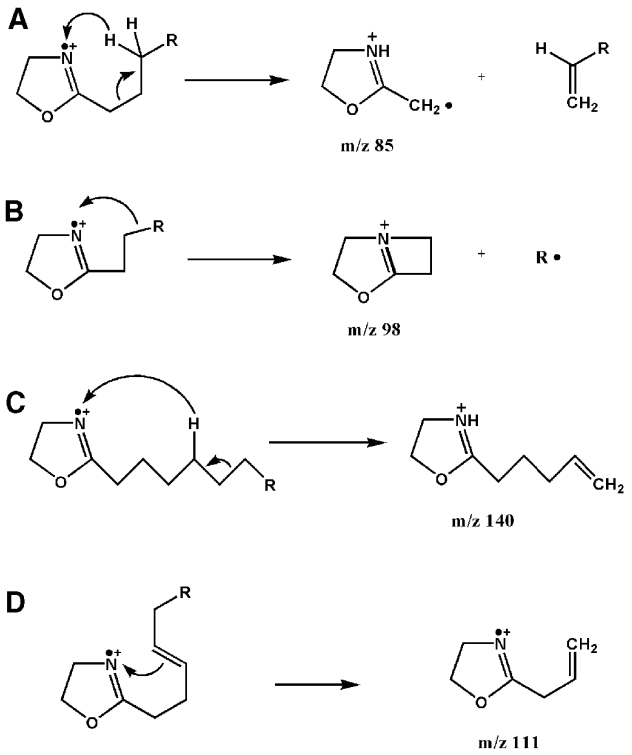
The oxazolines we synthesized were analyzed by ^{1}H-NMR and the presence of the cyclic oxazoline confirmed unequivocally based on the presence of signals for the four protons in its ^{1}H-NMR spectra that form two triplets with chemical shifts of 4.10 ppm (=N-C^{1}H^{1}H-CH$_2$-O) and 4.50 ppm (=N-CH$_2$-C^{1}H^{1}H-O) (δ-scale). The intensities were indicative of two protons each and had a spin-spin coupling constant J = 5 Hz. The data are in good agreement with earlier ^{1}H-NMR data on oxazolines (15, 16). We also emphasize that the signals for protons in the hydrocarbon chain were unchanged during formation of the oxazoline, indicating that no degradation occurred during the synthetic procedure. As an illustration of this, the ^{1}H-NMR spectra of docosahexaenoic acid and its oxazo-

line derivative were *a*) for docosahexaenoic acid: 0.95 (H-22; 3H, t), 1.9 (H-3; 2H, m), 2.07 (H-21; 2H, m), 2.40 (H-2; 2H, m), 2.50 (H-6; 2H, m), 2.84 (H-9,12,15,18; 8H, m), and 5.37 (H-4,5,7,8,10,11,13,14,16,17,19,20; 12H, m); and *b*) for the docosahexaenoic acid oxazoline: 0.95 (H-22; 3H, t), 1.9 (H-3; 2H, m), 2.07 (H-21; 2H, m), 2.43 (H-2; 2H, m), 2.50 (H-6; 2H, m), 2.84 (H-9,12,15,18; 8H, m), 4.10 (2H, =N-C^{1}H^{1}H-CH$_2$-O, t), 4.50 (2H, =N-CH$_2$-C^{1}H^{1}H–O, t), and 5.37 (H-4,5,7,8,10,11,13,14,16,17,19,20; 12H, m).

All of the oxazolines investigated were more polar than their corresponding 4,4-dimethyloxazolines and had lower R_f values on silica gel TLC, presumably due to the shielding effect of the methyl groups on the heterocycle. When *n*-hexane-ether (1:1, v/v) was used as the mobile phase, the R_f values for the oxazolines and the 4,4-dimethyloxazolines were 0.3 and 0.6, respectively. The oxazolines were less mobile than the corresponding 4,4-dimethyloxazolines during GC on a nonpolar phase (DB-5ms). The relative retention times of the methyl esters of fatty acids, their 4,4-dimethyloxazolines, and their oxazolines were in the ratio of 1.00:1.75:1.88.

Mass spectra of oxazolines

Oxazoline derivatives of fatty acids provide excellent mass spectra that frequently permit unequivocal structure assignments. The mass fragmentation of oxazolines has been studied in detail and parallels that of pyrrolidides and 4,4-dimethyloxazolines (1–3). The fragmentation pattern is relatively simple with the most prominent peaks due to ions containing nitrogen (i.e., an oxazoline ring), as illustrated for a generic 2-acyloxazoline in **Scheme 2**. The molecular ion $\{M\}^+$ is always accompanied by an $\{M\text{-}1\}^+$ ion, and in the case of most unsaturated fatty acids, the peak for the $\{M\text{-}1\}^+$ ion is at least as intense as that for the $\{M\}^+$ ion; indeed, as will be shown below, the peak for the $\{M\text{-}1\}^+$ ion is even more prominent with oxazolines of polyunsaturated fatty acids. Generally, two main fragment ion peaks are apparent in the spectra of oxazolines of straight-chain fatty acids: *i*) a prominent peak at *m/z* 85 formed as a result of the McLafferty rearrangement (Scheme 2, pathway a) and typically observed as the base peak in mass spectra of fatty acids with three or more double bonds; and *ii*) an even mass peak at *m/z* 98 typically seen as the base peak for saturated, monounsaturated, and dienoic fatty acids (Scheme 2, pathway b). These two peaks are the most prominent ones in almost all of the mass spectra we obtained. In the case of oxazolines of saturated fatty acids or fatty acids that have their first double bond at the Δ-9 position (i.e., oleic and linoleic acids), a peak for a fragment with *m/z* 140 is always present (Scheme 2, pathway c). Docosahexaenoic acid and docosaheptaenoic acids, both of which contain Δ4 double bonds, exhibit a peak at *m/z* 111 (Scheme 2, pathway d). The mass spectra of the oxazoline derivatives of fatty acids are structure specific, showing molecular ions and fragment peaks that permit facile identification of the location of double bonds. The spectra are essentially parallel to those of 4,4-dimethyloxazoline derivatives, with steps of 14 *m/z* between carbon atoms at saturated bonds and 12 *m/z* between carbon atoms of double bonds.



Scheme 2. Structures and derivation of the most prominent ions in the mass spectra of fatty acid oxazolines.

One of the features of 4,4-dimethyloxazolines of fatty acids is the presence of two methyl groups attached to the oxazoline ring. The presence of these methyl groups complicates data interpretation in that the peak at *m/z* $\{M\text{-}15\}^+$ can be explained as either a loss of a methyl group from the oxazoline ring or loss of a terminal or branched methyl group (6); this is a particular complication when interpreting data from 4,4-dimethyloxazolines of iso- and anteiso-isomers of fatty acids found in microorganisms (17). On the other hand, the presence of methyl groups and the ease with which they are eliminated from the oxazoline ring lead to formation of a peak at *m/z* $\{M\text{-}15\}^+$ of relatively high intensity that can serve as an additional confirmation of the correct value of *m/z* for the molecular ion. In the case of oxazoline derivatives, the ion with *m/z* $\{M\text{-}15\}^+$ can only originate from the loss of a terminal methyl group from the fatty acid. We have observed that the ratio of the intensities of the $\{M\text{-}15\}^+$ peaks of 4,4-dimethyloxazolines compared with oxazoline derivatives is about three, suggesting that the major source of the $\{M\text{-}15\}^+$ peak with 4,4-dimethyloxazolines the ring methyl groups. The lack of methyl groups in the ring of oxazoline derivatives eliminates the problem of the formation of the 5,5-dimethyloxazoline isomer and resulting "shadow" peaks during capilllary GC.

Mass spectra of 2-acyloxazolines derived from saturated and monounsaturated fatty acids

Palmitic, stearic, arachidic, and behenic acids were converted to their corresponding oxazolines as described

above (Scheme 1), with THF as the solvent in all cases. The oxazoline of stearic acid has a typical spectrum of an oxazoline of a saturated fatty acid (data not shown). Intense peaks at *m/z* 85, 98, 112, and 140 result from the McLafferty rearrangment or rearrangements involving reciprocal hydrogen transfer (Scheme 2). Peaks produced in this way include the C2, C5, and C6 fragments, respectively, from the original fatty acid. An even-mass homologous series *m/z* 98+14n (*m/z* 140, 154, 168, 182, etc.) occurs with regularity up to the molecular ion.

All of the oxazolines derived from monoenoic fatty acids showed distinct mass spectra characterized by an intense molecular ion peak (relative intensity 8–35%). All of the mass spectra were quite specific with respect to variation in the position of the double bond. In comparing the spectrum of the oxazoline derived from oleic acid with that of the oxazoline from stearic acid (data not shown), there was a shift in the peaks in the homologous series of ions by 2 amu toward the low-mass region that established the location of the site of unsaturation; for example, in the spectrum of the oxazoline of oleic acid, a mass difference of 12 amu was detected between *m/z* 168 (C_{8-9}) and 180 (C_{9-10}). Thus, the empirical rule formulated originally in studies of 4,4-dimethyloxazoline derivatives can be applied to oxazoline derivatives as well (9).

Mass spectra of 2-acyloxazolines derived from dienoic and trienoic fatty acids

In the spectrum of the oxazoline of linoleic acid, two clusters of peaks can be distinguished clearly (**Fig. 1**). The first cluster, with two prominent peaks at *m/z* 208 and *m/z* 248, denotes an ω6 double bond, and the second cluster, with peaks at *m/z* 154 and *m/z* 194, denotes a Δ9 double bond. A prominent molecular ion of *m/z* 305 and corresponding peaks with *m/z* 290, 276, 262, and 248 forming even-mass homologous ions are characteristic of the mass spectra of oxazoline derivatives of ω6 dienoic fatty acids.

The spectrum of the oxazoline of α-linolenic acid provides a good example of the applicability of oxazolines to structural investigations of polyunsaturated fatty acids (**Fig. 2**). Despite the degree of unsaturation, the mass spectrum is an informative one and can be explained easily. In the spectrum, an intense molecular ion (*m/z* 303) is still present, and three clusters at *m/z* 154–194, *m/z* 194–234, and *m/z* 234–274, with pairs of peaks at *m/z* 168–180, 208–220, and 248–260, and with an increment of 12 amu, establish the positions of the three double bonds.

Mass spectra of 2-acyloxazolines derived from highly unsaturated fatty acids

The structures of the oxazolines of arachidonic, eicosapentaenoic, and docosahexaenoic acids, three highly unsaturated fatty acids having classical methylene interrupted double-bond structures, were investigated. Despite the fact that more complicated spectra were observed with these oxazolines, a high degree of specificity was retained with respect to the fragmentation patterns. The mass spectrum of the oxazoline of docosahexaenoic acid is shown in **Fig. 3**. The peak for the $\{M\text{-}1\}^+$ ion rather than the one for the molecular ion is the dominant peak. This feature is even more prevalent in mass spectra of 4,4-dimethyloxazoline derivatives, but can also be distinguished clearly with the oxazoline derivatives of highly unsaturated fatty acids. In the spectrum of the oxazoline of docosahexaenoic acid, five clusters of ions are seen at *m/z* 124–164, *m/z* 164–204, *m/z* 204–244, *m/z* 244–284, and *m/z* 284–324

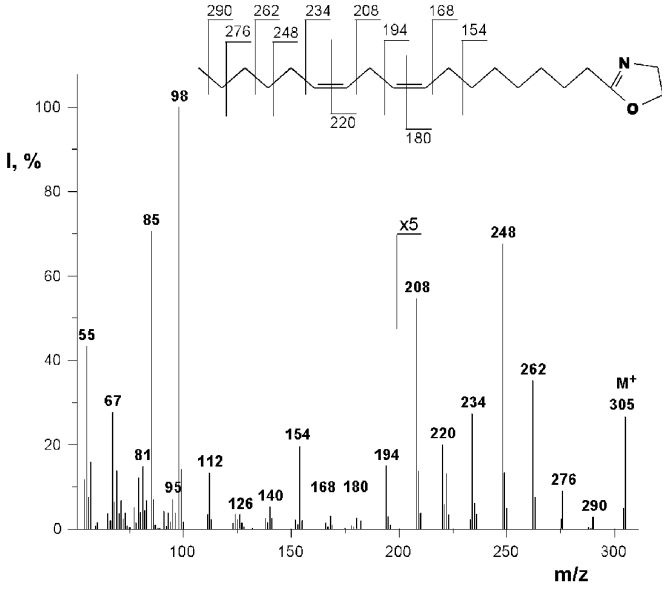


Fig. 1. Mass spectrum of the oxazoline of linoleic acid. The 2-acyloxazoline of linoleic acid was synthesized and then analyzed by gas chromatography-mass spectrometry (GC-MS) as detailed in the text.

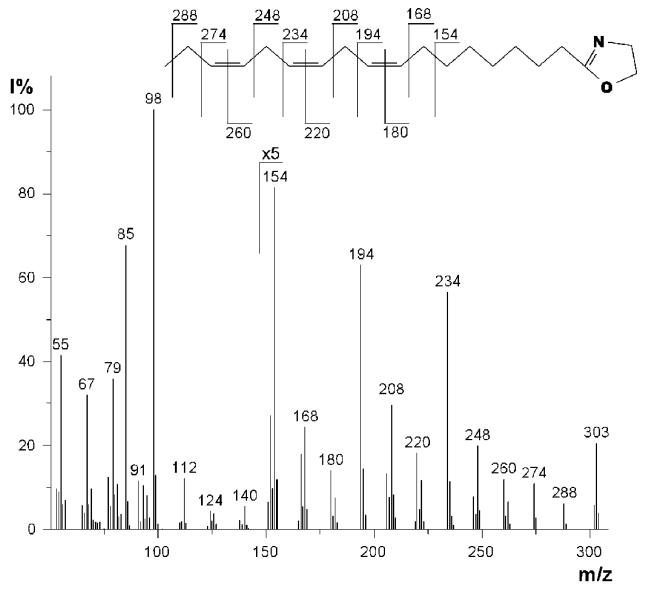


Fig. 2. Mass spectrum of the oxazoline of α-linolenic acid. The 2-acyloxazoline of α-linolenic acid was synthesized and then analyzed by GC-MS as detailed in the text.

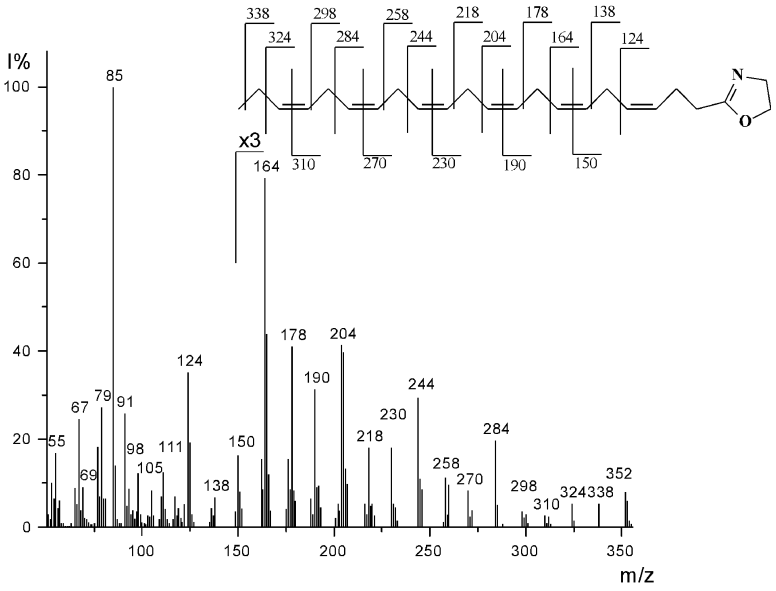


Fig. 3. Mass spectrum of the oxazoline of docosahexaenoic acid. The 2-acyloxazoline of docosahexaenoic acid was synthesized and then analyzed by GC-MS as detailed in the text.

(Fig. 3); the corresponding pairs of peaks, with a difference in 12 amu at *m/z* 150–138, *m/z* 190–178, *m/z* 230–218, *m/z* 270–258, and *m/z* 310–298, show the positions of five remote double bonds. The position of the Δ4 double bond can be found taking into account the fragmentation rule suggested by Spitzer (3) (a peak at *m/z* 111 characteristic of fatty acids having Δ4 double bonds).

We recently synthesized several highly unsaturated, polyconjugated fatty acids, including 4E,6E,8E,10E,13Z,16Z,19Z-docosaheptaenoic acid. The structure of this fatty acid has been confirmed using several independent techniques (unpublished observations). Shown in **Fig. 4** is the mass spectrum of the oxazoline of 4E,6E,8E,10E,13Z,16Z,19Z-docosaheptaenoic acid. A prominent molecular ion is present at *m/z* 351. The spectrum can be separated into two parts. The first part, located in the higher-mass region, contains three clusters at *m/z* 202–242, *m/z* 242–282, and *m/z* 282–322 that represent three methylene interrupted double bonds in the ω3 position (i.e., fragments $\{M\text{-}15\}^+$ at *m/z* 336, and $\{M\text{-}29\}^+$ at *m/z* 322). The second part of the spectrum, in the lower-mass region, with the *m/z* below 200, represents a very complicated fragmentation of the molecule at sites of conjugation. While the spectrum is not wholly definitive in the lower-mass region, prominent peaks are clearly seen for ions at *m/z* 85, 98, 111 (Δ4 double bond), and 141. It has not been possible previously to prepare dimethyloxazoline derivatives of this type of conjugated fatty acid, and methyl esters of conjugated fatty acids do not yield informative mass spectra.

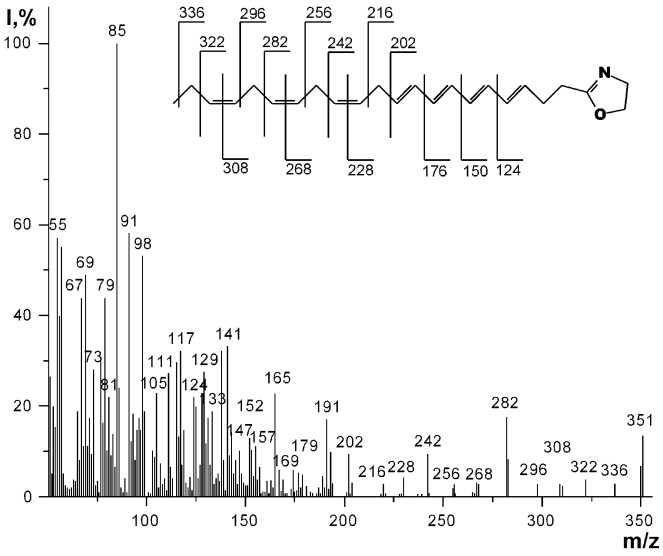


Fig. 4. Mass spectrum of the oxazoline of 4E,6E,8E,10E,13Z,16Z,19Z-docosaheptaenoic acid. The 2-acyloxazoline of 4E,6E,8E,10E,13Z,16Z,19Z-docosaheptaenoic acid was synthesized and then analyzed by GC-MS as detailed in the text.

Conclusions

We have developed a method for the preparation of 2-acyloxazolines that is mild enough to be used with highly-unsaturated, highly-conjugated, and otherwise relatively unstable fatty acids that is also applicable to the preparation of 4,4-dimethyloxazolines and benzoxazoles. The oxazoline derivatives of fatty acids we have analyzed yield mass spectral fragmentation patterns that are useful for determining the double-bond positions in highly unsaturated and conjugated fatty acids. Together, oxazoline, 4,4-dimethyloxazoline and benzoxazole derivatives are structurally complimentary and diagnostic in that they provide mass spectra with predicted shifts of 28 amu and 48 amu between fragments of the same structure derived from the oxazoline, 4,4-dimethyloxazoline, and benzoxazole derivatives, respectively.

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